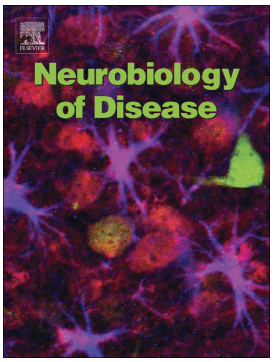


# Effects of α-synuclein on axonal transport

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***Title:*** *Effects of α-synuclein on axonal transport*

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***Abstract*** Lewy bodies and Lewy neurites composed primarily of α-synuclein characterize synucleinopathies including Parkinson's disease (PD) and dementia with Lewy bodies (DLB). Despite decades of research on the impact of α-synuclein, little is known how abnormal inclusion made of this protein compromise neuronal function. Emerging evidence suggests that defects in axonal transport caused by aggregated α -synuclein contribute to neuronal dysfunction. These defects appear to occur well before the onset of neuronal death. Susceptible neurons in PD such as dopamine neurons with long elaborate axons may be particularly sensitive to abnormal axonal transport. Axonal transport is critical for delivery of signaling molecules to the soma responsible for neuronal differentiation and survival. In addition, axonal transport delivers degradative organelles such as endosomes and autophagosomes to lysosomes located in the soma to degrade damaged proteins and organelles. Identifying the molecular mechanisms by which axonal transport is impaired in PD and DLB may help identify novel therapeutic targets to enhance neuron survival and even possibly prevent disease progression. Here, we review the evidence that axonal transport is impaired in synucleinopathies, and describe potential mechanisms by which contribute to these defects.

***Abbreviations:***

Dementia with Lewy bodies (DLB), Lewy bodies (LB), Lewy Neurites (LN), Locus coeruleus (LC), Parkinson's disease (PD), substantia nigra pars compacta (SNpc)

***Lewy neurites predominate in synucleinopathies***

The proteinaceous inclusions known as Lewy Bodies (LBs), which characterize Parkinson's disease (PD) and Dementia with Lewy bodies (DLB), were first described by Fritz Heinrich Lewy in 1912 (Lewy, 1912). LBs are eosinophilic, spherical, or reniform dense inclusions in the neuronal soma. Lewy neurites (LNs) found in brains of PD and LBD patients were also described by Lewy. These inclusions have received less attention than LBs because they are not easily stained with acidophilic dyes. The first study to appreciate the density of LNs utilized immunohistochemistry for ubiquitin, a protein modification found in LBs and LNs, and revealed that LNs appear as a dense network in the hippocampus of DLB brains (Dickson et al., 1991). The discovery that α-synuclein is the main component of LBs and LNs (Spillantini et al., 1997) led to the development of sensitive α-synuclein antibodies, which revealed that Lewy neurites are a prominent feature of PD and DLB. These inclusions, which are far more abundant than LBs (Braak et al., 1999; Duda et al., 2002a; Braak et al., 2003), can appear as thick and club-shaped, short and stubby, or longer and thread-like. They appear throughout the nervous system including, but not limited to, cardiac sympathetic neurons, dorsal nucleus of the vagus nerve, substantia nigra pars compacta (SNpc), nucleus basalis, amygdala, hippocampus, and cortex.

Not only are LNs more abundant than LBs, their appearance precedes that of LBs (Braak et al., 1999; Braak et al., 2003). This is not surprising given that α-synuclein primarily resides at the presynaptic terminal (Maroteaux et al., 1988). Pathologic analyses of PD brains ranging from cases with mild pathology to severe pathology show, for example, that a plexus of LNs appears in the hippocampus at PD stage 3 of the evolution of PD pathology, whereas dense LBs only appear in the hippocampus at the latest stage 6 (Braak et al., 2003). The abundance of LNs

correlates with the development of symptoms. In addition, Lewy neurites are the best correlate of cognitive symptoms in DLB (Irwin et al., 2012). More sensitive immunohistochemical techniques to differentiate aggregated α-synuclein from monomeric alpha-synuclein (Kramer and Schulz-Schaeffer, 2007; Spinelli et al., 2014) show that the majority of α-synuclein aggregates localize to the presynaptic terminal and axons and correspond with a reduction in dendritic spines in the postsynapse (Kramer and Schulz-Schaeffer, 2007). Thus, identifying these early aggregates in presynaptic terminals and axons may provide an even better correlate of PD and DLB symptoms, and help reveal the mechanisms by which α-synuclein aggregate formation causes neuronal dysfunction.

***Defects in axonal transport caused by α-synuclein aggregates may precede neurodegeneration***

Pathological analyses of PD brains from over 50 years ago showed accumulations of vesicles along axons and near α-synuclein inclusion (Duffy P.E. and V.M., 1965; Forno and Norville, 1976; Watanabe et al., 1977; Hayashida et al., 1993). These early studies suggest that α-synuclein aggregates impair membrane traffic. Studies in multiple neurodegenerative disorders such as Alzheimer's disease, amyotrophic lateral sclerosis, and Huntington's disease implicate impaired axonal transport of vesicles as a major contributor to neurodegeneration(Chevalier-Larsen and Holzbaur, 2006; Hinckelmann et al., 2013; Millecamps and Julien, 2013). There are fewer studies implicating axonal transport defects as contributing to neurodegeneration in PD, but accumulating data suggest axonal transport defects precede degeneration (Chung et al., 2009; Chu et al., 2012; Lamberts et al., 2015). Understanding defects in neuronal function before neurodegeneration occurs is critical because therapeutic targets can be identified that can intervene before intractable cell death. One of the first studies to reveal pathologic α-synuclein

aggregates in axon terminals in the hippocampus of PD and DLB showed they are associated with accumulations of synaptic vesicle proteins (Galvin et al., 1999). This study suggested that blocked axonal transport in neurons containing α-synuclein aggregates could cause a "dying back" of the axonal projections. Subsequent to this study, levels of the molecular motors associated with axonal transport were shown to be reduced in PD brains (Chu et al., 2012). Levels of kinesin, which facilitates anterograde axonal transport of cargo from the soma to presynaptic terminal, were dramatically reduced in the SNpc of PD brains well before loss of dopaminergic neurons. Levels of components of the dynein complex, which facilitates retrograde transport from the terminal back to the soma, were also reduced but only in late stage PD brains. The abundance of motor proteins was particularly low in neurons containing α-synuclein aggregates. These findings support that α-synuclein aggregate induced alteration in motor proteins can precede neurodegeneration in PD.

Animal models of abnormal α-synuclein help further dissect the contribution of axonal transport defects to neurodegeneration because they allow careful analyses of the time course in which defects emerge. For example, independent studies utilizing adeno associated viruses (AAV) induced overexpression of the A53T- or A30P-disease associated mutations in α-synuclein show reduced levels of kinesin and dynein in neurons with aggregated α-synuclein(Chung et al., 2009; Chu et al., 2012). Eight weeks after induction of A53T- α-synuclein, there is a substantial reduction in levels of kinesin isoforms without changes in other synaptic proteins or dopamine neuron loss. Kinesin levels are reduced in the striatum, but are increased in the SNpc, suggesting the motors are trapped in the soma and cannot bind to microtubule tracks to facilitate anterograde transport along the axon. Loss of nigral neurons was not apparent until 17 weeks after induction

of A53T- α-synuclein, again supporting that defects in axonal transport emerge well before the onset of neuron death(Chung et al., 2009).

Some drawbacks of using *in vivo* animal models is that they do not provide the resolution to visualize axonal transport in live neurons, measure the kinetics of axonal transport, or easily dissect the molecular mechanisms that may contribute to potential defects. This is critical because axonal transport is a dynamic process. Distinct cargo show very different patterns of transport along the axon (Millecamps and Julien, 2013; Maday et al., 2014). For example, synaptic vesicle precursors travel predominantly in an anterograde direction from the cell body out to the presynaptic terminal. Late endosomes, autophagosomes, and signaling endosomes carrying neurotrophin receptors show predominantly retrograde transport from the presynaptic terminal back to the soma. Furthermore, transport of different cargos is mediated by distinct adaptor proteins. Kinesin motors facilitate anterograde transport; 38 kinesin superfamily members are expressed in the brain. Dynein, which facilitates retrograde transport, is a large motor complex composed of multiple subunits with multiple isoforms and requires activation by a large complex, dynactin. There are many distinct adaptors that interact with these molecular motors that regulate the specificity of cargo. For example, transport of mitochondria is regulated by the Mitochondrial Rho GTPase, (Miro), and kinesin-1(Macaskill et al., 2009; Wang and Schwarz, 2009). Axonal transport of signaling endosomes and late endosomes depends on dynein complexes specifically containing the intermediate chain IC-1B(Pfister, 2015). Axonal transport of these organelles is also controlled by Rab7 and Rab7 interactors such as and Snapin (Johansson et al., 2007; Cai et al., 2010). Dynein also forms a complex with adaptors Lis and NdeI, which play a role in late endosome axonal transport (Pandey and Smith, 2011). Overall,

there is an increasing list of scaffolding proteins and adaptors that regulate transport of specific cargo along axons.

Recently, it was shown that exposure of neurons to fibrils made from recombinant α-synuclein can robustly induce endogenous α-synuclein to form inclusions that resemble Lewy bodies and Lewy neurites found in diseased brains (FIGURE 1) (Volpicelli-Daley et al., 2011; Volpicelli-Daley et al., 2016). This is the first model that produces inclusions resembling LBs and LNs in primary neurons. This model provides the spatial resolution to determine subcellular neuronal compartments to which the inclusions localize, and provides the temporal resolution to examine inclusions from their initial formation to their spread throughout the neuron and consequent cell death. For example, after addition of fibrils to neurons, inclusions form first in axons (4-7 days after adding fibrils) and then spread to the soma and dendrites (10-14 days post-fibril addition), similar to findings from pathologic studies of PD brains(Volpicelli-Daley et al., 2011). Formation of inclusions in primary neurons also allows live cell imaging and quantitation of the kinetics of axonal transport of distinct organelles that move along the axon within milliseconds (Volpicelli-Daley et al., 2014). It was found that the presence of α-synuclein inclusions in axons at early time points, before neuron death, reduces velocities and mobilities of Rab7-positive late endosomes, TrkB neurotrophin receptors, and LC3-positive autophagosomes (FIGURE 2). However, axonal transport of mitochondria and synaptic vesicle precursors remain normal. In addition, electron microscopy analyses showed that α-synuclein inclusions do not fill the axonal cytoplasm or grossly disrupt the microtubule tracts. This suggests that the α-synuclein inclusions do not impair axonal transport by occluding the axon, nor do the inclusions cause a generalized disruption of transport proteins such as kinesin or dynein. Axonal transport of endosomal

organelles appear to be particularly affected by the presence of axonal Lewy neurite-like inclusions. However, it is possible that at longer time points after fibril addition, when the inclusions become more mature, transport of other organelles such as mitochondria may be impaired. Also, α-synuclein has been shown to act as a microtubule dynamase (Cartelli et al., 2016) and more sensitive biochemical techniques may reveal if the inclusions impact microtubule dynamics, causing disrupted axonal transport. Furthermore, it will be of great interest to determine if neurons particularly susceptible in PD, such as dopamine neurons, are more sensitive to defects in axonal transport.

***Potential mechanisms by which α-synuclein aggregates impair axonal transport***

The mechanisms by which α-synuclein aggregates impair axonal transport are unknown. Some rare neurodegenerative diseases are caused by mutations in motor proteins themselves such as (Zhao et al., 2001) in Charcot Marie Tooth disease, or KIF5A in spastic paraplegia (Reid et al., 2002). Mutations in motor proteins have not been identified in PD. However, it is possible that the α-synuclein aggregates may selectively sequester or impair the function of motor proteins and associated adaptors. Indeed, proteomics of purified Lewy bodies showed enrichment of dynein and dynactin (Xia et al., 2008). In addition, evidence from proteins implicated in other neurodegenerative diseases show this can occur. For example, huntingtin and huntingtin associated protein form a complex that regulates the motor activity of kinesin and dynein (Wong and Holzbaur, 2014). Polyglutamine expansions in huntingtin disrupt this complex and inhibit axonal transport of autophagosomes. The ALS associated mutation SODG93A inhibits retrograde transport of TrkB receptors (Klinman and Holzbaur, 2015). The dynein adaptor NdeI is hyperphosporylated in neurons expressing SDOG93A and inhibition of CDK5, which

phosphorylates NdeI, restores axonal transport of TrkB. Whether α-synuclein aggregates in PD impact distinct motor proteins and adaptors remains to be explored.

α-Synuclein selectively binds to membranes containing the acidic phospholipids phosphatidic acid, phosphatidylserine, or phosphoinositide (Davidson et al., 1998; Perrin et al., 2000). These lipids confer membrane identity to organelles throughout the cell. For example, phosphoinositide 4,5 bisphosphate PI(4,5)P2 is selectively enriched at the plasma membrane, PI(4)P at synaptic vesicles, PI(3)P at endosomes, and PI(3,5)P2 at late endosomes (Volpicelli-Daley and De Camilli, 2007). Association of α-synuclein with membranes containing acidic phospholipids has been shown to trigger its conversion into amyloid aggregates. Although α-synuclein has classically been placed at synaptic vesicles, it also localizes to endosomes within the axon (Boassa et al., 2013). It is possible that in the intact neuron, α-synuclein selectively associates with membranes of distinct composition such as PI(4)P, PI(3)P, or PI(3,5)P2 containing synaptic vesicles, endosomes, and late endosomes. The inclusions could grow from these membranes (Galvagnion et al., 2015)and also selectively trap these organelles. Indeed, our electron microscopy analyses show what appear to be late endosomes/multivesicular bodies trapped within the filamentous inclusions (FIGURE 3), similar to pathologic studies from PD brains showing accumulations of vesicles within LBs.

The microtubule binding protein, tau, may also play a role in axonal transport defects in neurons with α-synuclein aggregates. Genetic variation in tau (gene symbol MAPT) consistently produces a very strong signal in genome wide association studies (GWAS) evaluating risk factors for sporadic for PD. PDGENE.org, which tracks GWAS associations, currently assigns a

p value of less than $6x10^{-49}$ for the linkage between tau and PD, second only to synuclein(Tobin et al., 2008; Satake et al., 2009; Simon-Sanchez et al., 2009). Aggregates of α-synuclein and tau co-occur in the same neuron, particularly in DLB (Duda et al., 2002b; Ishizawa et al., 2003; Fujishiro et al., 2008). In addition, PD patients with dementia have a higher burden of aggregates of tau, called neurofibrillary tangles, in the cortex of their brains than patients with PD who do not have dementia (Irwin et al., 2012). Increased levels of tau inhibit kinesin and dynein motor activity (Dixit et al., 2008). Interestingly, absence of tau restores axonal transport in Alzheimer's disease models (Vossel et al., 2015). Thus, aggregates of tau may also contribute to α-syn inclusion induced defects in axonal transport.

Aggregates of α-synuclein have also been shown to selectively sequester Rab proteins. There are over 70 Rab proteins, which are small GTP binding proteins that associate with distinct organelles, conferring organelle identity and directing transport to target organelles (Zhen and Stenmark, 2015). For example, Rab1 directs transport from the endoplasmic reticulum to the Golgi, Rab5 directs transport from clathrin coated vesicles to early endosomes, and Rab7 mediates traffic from early endosomes to late endosomes. In yeast cells, formation of α-synuclein aggregates promotes accumulation of vesicles containing specific Rabs and causes toxicity (Gitler et al., 2008). Overexpression of select Rabs protects against toxicity produced by α-synuclein aggregates. In addition, Rab1A, Rab8A (a close paralogue to Rab1) and Rab3A, selectively, protect against dopamine neuron cell loss in Parkinson's disease animal models. Thus, in neurons, α-synuclein inclusions may promote the accumulation of distinct vesicles and organelles identified by select Rab GTPases. It would be of great interest to determine if overexpression of these Rabs prevents toxicity in neurons with fibril induced inclusions as well.

Leucine rich repeat kinase (LRRK2) may also play a role in axonal transport. Mutations in LRRK2 are the most common cause of familial Parkinson's disease. Mutant LRRK2 forms a complex with Miro (Hsieh et al., 2016), a mitochondria associated small GTPase that along with other adaptors facilitates both anterograde and retrograde axonal transport of mitochondria. Mutant LRRK2 disrupts this complex. In addition, LRRK2 mutations have been shown to inhibit axonal transport of mitochondria by preferentially binding to deacetylated microtubules (Godena et al., 2014). Several studies also implicate LRRK2 in traffic of endosomes and autophagosomes (Friedman et al., 2012; Schapansky et al., 2014). LRRK2 loss of function mutants reduce the abundance of axonal Rab7-positive endosomes, suggesting it may play a role in late endosome axonal transport(Kuwahara et al., 2016). The PD-associated mutation, G2019S-LRRK2, shows enhanced association with dynein heavy chain, suggesting that LRRK2 play a role in dynein-mediated transport of endosomes and autophagosomes (Sen et al., 2009). Interestingly, select Rab GTPases have been identified as bona fide LRRK2 substrates (Steger et al., 2016). These include Rab1, Rab3, and Rab8, which protect against α-synuclein aggregate induced toxicity(Gitler et al., 2008). Thus, several lines of evidence are converging to implicate that LRRK2 and α-synuclein, two of the most common genes associated with PD, play roles in traffic of select organelles.

***What are the potential consequences of impaired axonal transport?***

Studies using toxin models of PD, e.g. MPTP and 6-hydroxydopamine, showed that the trophic factors GDNF or neurturin protect against neurodegeneration. However, clinical trials of neurturin failed to produce significant benefits (Olanow et al., 2015). Impaired axonal transport

may have contributed to the inability of neurotrophic factors to promote neuron survival or enhance outgrowth. Axonal transport of neurotrophin receptors in endosomes (called signaling endosomes) is required to deliver signaling molecules to the neuronal soma where they can translocate to the nucleus to activate gene transcription factors important for neuronal differentiation and survival. Disrupted axonal transport could potentially impair growth factor signaling leading to neurodegeneration. Formation of Lewy-neurite like inclusions in primary neurons impairs transport of the neurotrophin receptor TrkB and causes an accumulation of signaling molecules in the cytoplasm(Volpicelli-Daley et al., 2014). In addition, GDNF treatment is not protective in the AAV- α-synuclein model, again suggesting that pathologic α-synuclein disrupts neurotrophic signaling (Decressac et al., 2011; Decressac et al., 2012). Overexpression of the nuclear receptor, Nurr1, is protective. Direct expression of Nurr1 in the nucleus bypasses the requirement of retrograde axonal transport of the GDNF activated Ret receptor. Thus, direct activation of nuclear receptors may be an alternative therapeutic strategy rather than ligand-induced activation of receptors.

Retrograde transport of degradative organelles such as endosomes and autophagosomes is required for targeting proteins and damaged organelles to lysosomes located in the soma for degradation (Maday and Holzbaur, 2016). Impaired retrograde transport of these organelles may prevent targeting of aggregated α-synuclein to lysosomes (Sacino et al., 2016). This could potentially result in a pathogenic positive feedback loop in which defective transport of degradative organelles prevents targeting of α-synuclein aggregates to lysosomes, augmenting inclusion formation. Alleviating transport defects may enhance α-syn aggregate degradation and reduce inclusion formation. For example, expression of molecules selectively involved in

retrograde axonal transport, such as DIC-1B, Snapin, or LIS1, may enhance retrograde axonal transport. Recently, it was shown that aberrant increased activity of CDK5 in neurodegenerative models increases phosphorylation of the LIS1 complex and inhibits transport of dynein-mediated retrograde transport of endosomes and autophagosomes. Inhibition of CDK5 with roscovatine restores these transport defects (Klinman and Holzbaur, 2015). Roscovatine is already in clinical development for the treatment of cancer and thus may be a potential therapeutic target to alleviate axonal transport defects, improve growth factor signaling, and consequently induce axonal outgrowth or enhance neuronal survival.

***Axonal transport and spread of α-synuclein inclusions.***

Axonal transport may also contribute to the spread of pathological α-synuclein across interconnected networks. First, staging of Lewy pathology in PD suggests that pathology can spread retrogradely along the vagus nerve (Braak et al., 2003). Lewy pathology also appears early in the disease process in the olfactory bulb and is thought to contribute to anosmia, a common symptom of PD. Synthetic fibrils injected into the mouse olfactory bulb are taken up by mitral cells and induce endogenous α-synuclein to form Lewy body and Lewy neurite inclusions(Rey et al., 2016). As early as one month after injections, inclusions appear in piriform cortex, entorhinal cortex, amygdala, and olfactory nucleus. These brain areas receive direct projections from mitral cells but also send axonal projections to mitral cells. Thus, the spread of pathologic α-synuclein could occur in either an anterograde or retrograde manner along axons.

Experiments utilizing primary neurons plated in microfluidic chambers (which separate neuron soma/dendrites from axons) and live cell imaging show that α-synuclein fibrils can move in both

the anterograde and retrograde directions(Freundt et al., 2012). This system allowed visualization of fluorescent tagged α-synuclein fibrils, which could be seen to move in both anterograde and retrograde directions along axons. In addition, α-synuclein fibrils can be released into the extracellular space from axons or the somatodendritic domain. However, seeding and corruption of endogenously expressed α-synuclein by internalized fibrils occurs first in axons and inclusions appear in the soma and dendrites at later time points (Volpicelli-Daley et al., 2014). This is likely because α-synuclein concentrates at presynaptic terminals (Maroteaux et al., 1988). These findings would predict that since α-synuclein is primarily a presynaptic protein, seeding and corruption of endogenous α-synuclein by fibrils is more efficient in axons and that the spread of pathology, while it can occur in an anterograde direction, is more likely to occur in a retrograde direction along axonal projections.

This raises the question of how pathology could spread from the dorsal vagus complex to the SNpc as there are (Wang et al., 2014). However, locus coeruleus (LC) neurons in the pons send projections to the dorsal vagus complex in the medulla and SN neurons in the midbrain project to the LC in the pons. Furthermore, LC neurons have Lewy pathology and degenerate in Parkinson's disease suggesting that SNpc projection neurons may take up aggregated α-synuclein from the LC. Overall, α-synuclein pathology likely initiates at axon terminals and spreads in a retrograde fashion along the axon. In neurons with normal lysosome function, the aggregates can be degraded and destroyed (Sacino et al., 2016). However, in neurons with abnormal lysosome function, impaired degradation would cause a buildup of toxic fibrils or oligomers that can be released and taken up by neighboring axon terminals. Future studies however are required to support this hypothesis.

***Conclusions***

Parkinson's disease is characterized by selective degeneration of neuronal subpopulations. Despite decades of research, little is known about potential defects in neuron function preceding neurodegeneration. Understanding the impacts of α-syn aggregation on neuronal function could help develop therapeutic interventions before the neurons undergo intractable degeneration. Defects in axonal transport could contribute to neuronal demise by inhibiting transport of signaling molecules critical for differentiation and survival or by impairing degradative processes causing a build of toxic, misfolded synuclein. Axonal transport also likely contributes to the spread of pathogenic α-synuclein throughout interconnected pathways in the brain. The mechanisms by which axonal transport is impaired in PD remain to be discovered, but could help identify novel therapeutic targets to halt the progression of this devastating disease.

***Figure Legends***

**Figure 1.** Four μL of 350 μM mouse α-synuclein fibrils were sonicated and injected into the rat SN. An equal concentration of monomeric α-synuclein was injected into the contralateral side. One month later, the rat was perfused and immunohistochemistry was performed using an antibody to pS129-α-synuclein. Minimal background staining was seen in the monomer injected side (Panels $A_1$, $B_1$, $C_1$). Inclusions were seen in the SN of the injected side ($B_2$). Higher magnification images show diffuse cytosolic inclusions (plus sign), Lewy-neurite-like inclusions (arrow) and a spherical inclusion ($C_2$ and D). Adapted from Volpicelli-Daley LA, Abdelmotilib H, Liu Z, Stoyka L, Daher JP, Milnerwood AJ, Unni VK, Hirst WD, Yue Z, Zhao HT, Fraser K, Kennedy RE, West AB (2016) G2019S-LRRK2 Expression Augments alpha-Synuclein Sequestration into Inclusions in Neurons. The Journal of neuroscience : the official journal of the Society for Neuroscience 36:7415-7427.

**Figure 2.** Neurons were transfected with GFP-Rab7 and sonicated fibrils were added at DIV6 to induce inclusion formation from endogenous α-synuclein. Seven days later, a time point at which there are abundant inclusions in axons, but no cell death, live cell imaging was performed to analyze axonal transport of GFP-Rab7. The kymographs below the images of GFP-Rab7 in axons show distance traveled over time. The velocities of retrograde transport of GFP-Rab7 in neurons with axonal inclusions was significantly reduced. Adapted from Volpicelli-Daley LA, Gamble KL, Schultheiss CE, Riddle DM, West AB, Lee VM (2014) Formation of alpha-synuclein Lewy neurite-like aggregates in axons impedes the transport of distinct endosomes. Mol Biol Cell 25:4010-4023.

**Figure 3.** Primary hippocampal neurons were exposed to fibrils and fourteen days later were processed for electron microscopy using a primary antibody to pS129-α-synuclein and nanogold conjugated secondary antibody. Vesicles appear to accumulate near filamentous α-synuclein that are not visible on the electron micrograph presented. Scale bar= 500nm.

**Figure 4.** Cartoon illustrating the hypothesis that that impaired retrograde axonal transport of degradative organelles caused by aggregated a-syn causes a pathogenic positive feedback loop that amplifies inclusion formation.

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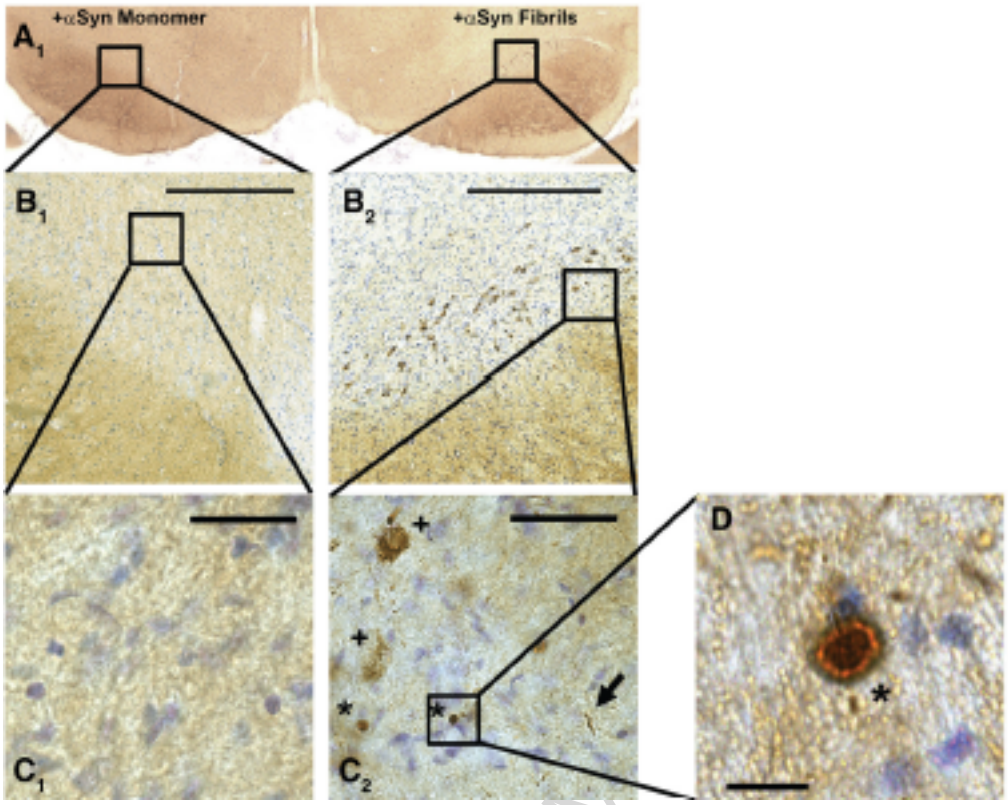


Figure 1

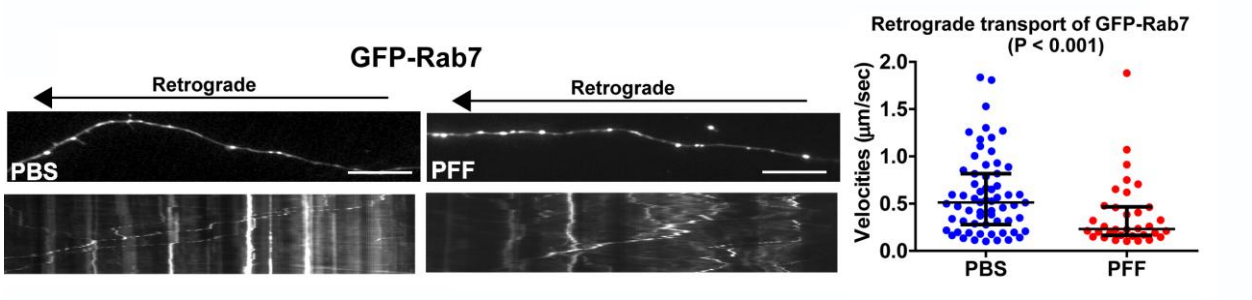


Figure 2

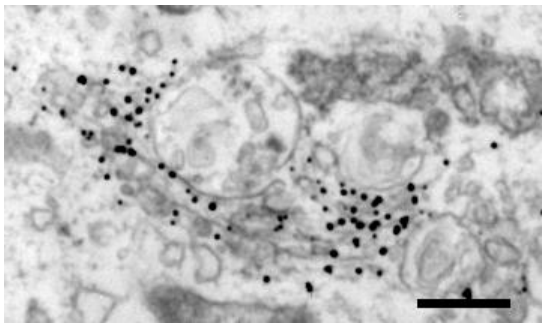

Figure 3

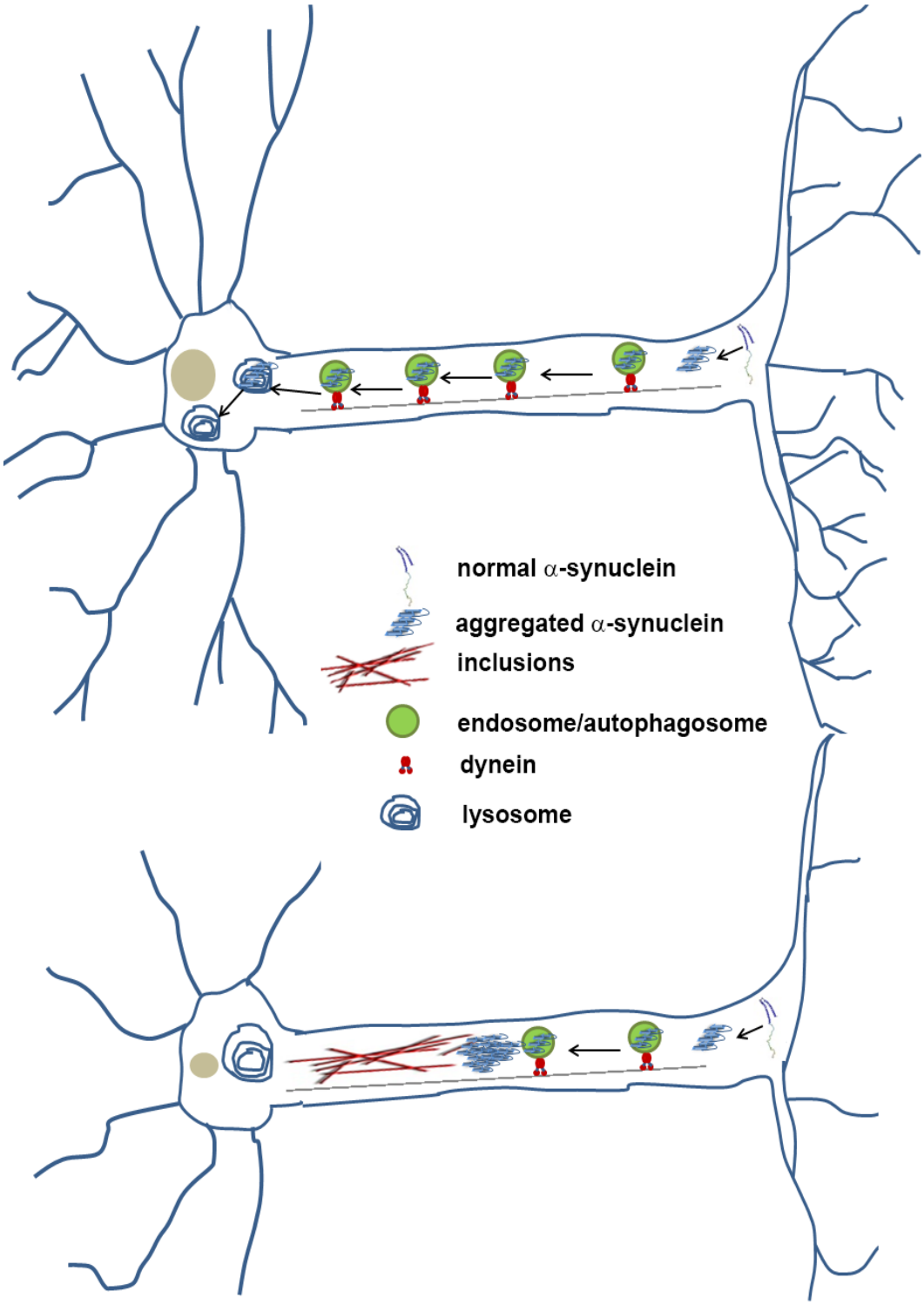


Figure 4